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Seminal biochemistry of mangrove crab, *Parasesarma plicatum* (Latreille, 1803)

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ABSTRACT

Biochemical studies have conducted in the male reproductive system of mangrove crab *Parasesarma plicatum* in order to provide information about sperm metabolism. The male reproductive system comprises of a pair of testes, vas deferens and accessory glands. In crabs, the sperm cells are atypical, non-motile and encapsulated in packets named spermatophores. Among the organic constituents, protein predominates in various regions of the reproductive system when compared with carbohydrates and lipids. The protein content of the seminal fluid differs in different zones of vas deferens. In our study, seminal carbohydrate exist as conjugated form as glycoprotein or muco substances, which significantly improve male fitness by increasing sperm storage. Seminal fluid consists of trace amounts of glycogen, ascorbic acid, protein bound hexose and inorganic ion such as calcium, sodium, potassium and magnesium. In the present study, the organic and inorganic constituents in the seminal plasma of *P. plicatum* may be involved in sperm viability during their prolonged storage within the male and female tract by providing a nutrient rich medium. In addition to this antibacterial role of accessory gland peptides also cannot be negated.
KEY WORDS: Seminal biochemistry, *Parasesarma plicatum*, spermatophores

INTRODUCTION

The sperm of decapod crustaceans are atypical in that they are aflagellate and non-motile [1,2,3,4,5,6]. In crab, the sperm masses become surrounded by seminal fluids secreted by the ductal and associated glands to become spermatophores [7,8]. Spermatophores are transferred to the seminal receptacles of the females at the time of copulation. The sperm will be stored for periods of time in female before oviposition [3,4,5,6,7,8]. The spermatophores internalized or may attach to the exoskeleton until the time of oviposition [9,10,11]. Free sperms have been observed in the lumen of the seminal receptacles or the spermatheca of the female crab [11] and the closed thelyca of penaeoidean shrimp, *Penaeus monodon* [12] after copulation.

In crustaceans, where external fertilization prevails, the semen plays a crucial role of protecting the sperm cells from desiccation and mechanical damage. For this reason, the spermatophores of decapods furnished with supporting devices. On the other hand, the groups where internal fertilization prevails, the spermatophores are simple. The role of spermatophore components and the seminal fluids in crustacean sperm protection and nutrition is unknown [13]. Few studies have been conducted on the nature of the chemical composition of the spermatophores and of the seminal fluid [14,15,16,]. However, more biochemical studies are necessary to determine how spermatozoa remain viable for a long time within the seminal receptacle or thelyca of females. This knowledge could be of immense value from the standpoints of commercial applications as it could help us in understanding the mechanisms involved in sperm preservation (including cryopreservation) and artificial insemination, to optimize aquacultural practices. The present chapter envisages the biochemical composition of semen secreted from the various regions of the male gonadal glands of the mangrove sesarmid crab, *P. plicatum*.

MATERIAL AND METHODS

Adult males of carapace width 1.6 cm to 2.2 cm were collected from Manakudy estuary (Lat. 8º4' N; Long. and 77°26' E) of Kanyakumari District, Tamil Nadu, India. On a weekly basis collections were handpicked and or

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made by bait. After examining the moult stages [17], crabs were reared in a laboratory in plastic cisterns and were fed ad lib on clam meat and (boiled) egg white. Inter-moult crabs used for biochemical studies.

Samples of anterior, middle and posterior (AVD,MVD and PVD)vas deferens and accessory gland (AG) were dissected out separately. The total TCA precipitable protein and ascorbic acid content was estimate using the Folin-Ciocalteu reagent by the procedure adopted by [18]. For the estimation of carbohydrates, tissues were extracted with 80% ethanol, the polysaccharides and oligosaccharides fractions were separated [19] and quantitatively estimated using the phenol-sulphuric acid [20]. The extraction of lipid done as per the method of [21] and the estimation followed as described by [22]. Inorganic ions such as sodium, potassium and magnesium were estimated flame photometrically. Calcium was estimated using Calcium kit (Sigma Diagnostics).

Characterization of Protein profile

The protein fractions of the vasal contents separated by Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) performed following the protocol [23]. The gel immersed in 5 ml of staining solution (200 mg Coomassie brilliant blue R250+50 ml MeOH (Methyl hydroxide) + 7 ml acetic acid + 43 ml distilled water and was allowed to stain for 4 hours at room temperature. The stain of gel removed and destained with acetic acid and methyl hydroxide solution (7 ml acetic acid + 30 ml Methyl hydroxide + 63 ml distilled water). The gel was stored in 7% acetic acid; the bands visualized under UV transilluminator.

RESULTS

Testis

The contents of the testis of *P. plicatum* appear transparent white, its pH being 7.0. Protein is found to be dominant over the other organic and inorganic components (3.94 ± 1.16 mg/100 mg tissue weight). The quantities of polysaccharide and oligosaccharide fractions are about 1.39 ± 0.47 and 0.80 ± 0.24 mg/100 mg tissue weight respectively. Other organic components present in the semen of *P. plicatum* are mucoprotein (0.48 ± 0.25 mg/100 mg tissue weight), protein bound hexose (0.32 ± 0.17 mg/100 mg tissue weight), and lipid (0.84 ± 0.17 mg/100 mg tissue weight). Glycogen, glucose and ascorbic acid are present in traces. The organic components present in the luminal contents of the testis of *P. plicatum* represented in the Table 1. In the testis inorganic ions such as sodium, potassium, magnesium and calcium are detected (Table 2).

Vas deferens

The seminal fluid of *P. plicatum* appears viscous and milky white in colour, and has slightly alkaline pH (7-8). Water is the major constituent in AVD and MVD. Seminal secretions of the whole VD constitute mainly protein (28.62 ± 4.12 mg/100 mg tissue weight). The polysaccharide and the oligosaccharide fractions, and the lipid constitute about 4.6 ± 0.64, 2.82 ± 0.28 and 1.76 ± 0.13 mg/100 mg tissue weight respectively. The levels of mucoprotein, protein-bound hexose and glycogen were only in moderate amounts, while glucose and ascorbic acid were present in traces. The organic and the inorganic components of the vas deferens of *P. plicatum* represented in the Tables 1 and 2 respectively. Regional (AVD, MVD) discrepancies in the major biochemical constituents of the seminal fluid of *P. plicatum* are represented graphically (Fig.1).

Accessory glands

The accessory gland content of *P. plicatum* is milky-white with slightly alkaline pH (7-8). Protein forms the major component (21.6 ± 2.01 mg/100 mg tissue weights) of the accessory gland, while polysaccharide and oligosaccharide fractions constitute 2.48 ± 0.77mg/100 mg tissue weight and 1.68 ± 0.26 mg/100 mg tissue weight respectively. Further, protein bound hexose (1.06 ± 0.28 mg/100 mg tissue weight), muco-protein (1.62 ± 0.31 mg/100 mg tissue weight), glycogen (0.28 ± 0.16 mg/100 mg tissue weight), glucose (0.04 ± 0.03 mg/100 mg tissue weight) and ascorbic acid (0.024 ± 0.008 mg/100 mg tissue weight) are found to be present in moderate levels (Table.1). The inorganic ions like sodium, potassium, magnesium and calcium have estimated and represented in the Table (2).

Electrophoretic analysis

Electrophoretic analysis of the testis of *P. plicatum* revealed the occurrence of a single polypeptide band with a molecular weight of 13,134.328 kDa (Fig 2 and 4). 10 bands were resolved in both VD and AG. The vasal secretions (VD) revealed 3 major bands with molecular weights ranging between 8,333 and 51,188 kDa respectively. The polypeptide bands, 5, 6 and 7 were with the same molecular weight but differs in Rf values. The molecular weight of other peptide bands ranges between 61,570 and 241,891 kDa respectively. The detailed

description of the peptide band pattern and molecular weight were represented in Fig (3). In AG, of the 10-peptide bands, 4 were major and 6 were minor peptide bands. Bands No., 4, 5 and 6 were with same molecular weight but with different Rf value (Fig 2 and 6) (Table. 4, 5 and 6).

DISCUSSION

The colour of the seminal fluid in *P. plicatum* (milky white) conforms to the general pattern described in other crustaceans [24,25,26,27]. The biochemical analysis of the vas deferens of *P. plicatum* reveals the predominance of protein, followed by carbohydrate and lipid, as has been reported in other brachyurans [6,10,21,23,24,25,26]. The investigations on the spermatophore-carrying seminal plasma of the brachyuran crabs have also indicated the predominance of protein among other components such as carbohydrates and lipids [9,16,27,28].

The protein content of the seminal plasma of *P. plicatum* differs in various zones of VD like what has been reported in other brachyurans *viz., P. hydrodromous* [25], *S. serrata* [9,27,] *M. messor* and *S. quadratum* [10]. In *P. plicatum*, the quantity of total protein in the AVD is significantly less ($t = 3.18$; $P < 0.001$) than that of the MVD. The increased protein content in the MVD may be due to its enormous size with additional diverticulae-like structures [4,5,6]. Suganthi and Anilkumar [29] suggested that the wall of the MVD may secrete substantial amount of protein, or it may be that the protein profile of MVD is the cumulative quantity amassed from the AVD and MVD.

The exact role of protein in the seminal fluid of brachyurans is still enigmatic. A portion of protein used as organic reserve for the sperm survival both within the male tract (pre-copulative) and after reaching the spermatheca (post-copulative). Another possibility is that, it may offer mechanical support to the sperm by forming a constituent part of the spermatophore wall substance and/or a part of the seminal plasma. There have been several reports on the antibacterial activity of the seminal plasma in crustaceans [29,30,31,32,33,34,35,36] against a wide range of microorganisms. This suggests that there might be some components in the seminal plasma of crabs responsible for offering resistance against the invading bacteria or other pathogenic microorganisms. Hoq et al. [35] found that the components responsible for antibacterial activity were proteins. The aforementioned reports tempt us to suggest that the proteins and the peptides present in the seminal fluid of the test crab may also have such an antimicrobial role.

The seminal carbohydrates found to be present in moderate levels in *P. plicatum*, a situation comparable with those of *Paratelphusa hydrodromous* [24], *Ocypode platytarsis* [25], *Metopograpsus messor* and *Sesarma quadratum* [10] and *Diogenes costatus* [26]. The polysaccharide and the oligosaccharide fractions show a tendency of increase in VD than in testis and the AG. It is apparent that, at least the spermatozoa could utilize a portion of the carbohydrate resource, while encased within the spermatophore wall. In this context, it may be worth recalling that the brachyuran spermatozoa are suggested to rely considerably on anaerobic glycolysis, while stored within the spermatophore [27,29].

The constituent of lipid in the crustacean semen has so far received only very scanty attention. Less amounts of lipid in the VD of *P. plicatum* possibly suggest that the sperm rely on anaerobic metabolism and such involvement of lipids in sperm metabolism is more necessary during their prolonged storage within the female spermatheca. Lipids and fatty acids in the seminal fluids of insects are male specific and in some cases, mediate the reproductive behavior of the female after copulation [37].

The glycogen of the vas deferens of *P. plicatum* may function as a storage reserve that could meet the energy requisite of the stored spermatozoa. Presence of glycogen in the seminal plasma of *O. platytarsis* [25], *M. messor* and *S. quadratum* [10] reported to act as reserve for the stored sperm [38].

Ascorbic acid is present in detectable amounts in the seminal fluid of *P. plicatum*, as reported in other brachyurans (10]. Ascorbic acid has been suggested to perform several functions such as, the degradation of tyrosine and phenyl alanine [39], protection of -5 H groups and thiol groups against heavy metals [40], synthesis of steroids in the male reproductive system [41] and induction of mating in *B. balanus* [40].

Seminal fluid of *P. plicatum* shows the presence of inorganic ions such as calcium, sodium, potassium and magnesium. In *P. plicatum*, the quantity of sodium is higher than that of other inorganic ions like that of *S. quadratum* [10], whereas in *M. messor,* potassium present in higher amounts. The presence of inorganic ions like Na, K, Cu, Mn, Mg and Zn has reported in the seminal plasma and spermatophores of *Pachygrapsus crassipes* [42].

Presence of sodium and potassium ions in the animal body functions in the regulation of osmotic pressure, ionic regulation, acid-base balance and catalytic agent for certain enzymatic activities. These inorganic ions in the semen are required for the high metabolic activity and utilization of substrates of sperm cells [43]. In the present study, the organic and inorganic constituents in the seminal plasma of *P. plicatum* may be involved in sperm viability during their prolonged storage within the male and female tract by providing a nutrient rich medium and an extra cellular physiological saline with adequate ionic strength for keeping the osmotic balance at a constant rate.

The major organic component of the accessory gland (AG) of *P. plicatum* is protein. From previous reports [25,29] and from the present study, it is obvious that the AG secretions of brachyuran crabs neither contribute to the spermatophore matrix nor play any role in spermatophore wall formation. However, may have an alimentary role that facilitates long-term sperm storage within the male duct. Upon reaching the spermatheca, these protein secretions may dissolve the spermatophore wall.

P. plicatum lives in crevices, burrows in the estuarine region, and exposed to coastal waters containing hosts of microorganisms. Obviously, the male and the female genital orifices of crabs are exposed to the external environment containing several microorganisms, deleterious to fertility [34,44]. It could presume that the accessory gland proteins of the crab might also contain substances that have an antibacterial effect, in order to protect the reproductive tract against microbial infection. Polysaccharide and oligosaccharide fractions of accessory gland secretion contribute to the metabolic energy for the survival of spermatozoa within the female tract on a long-term basis, as the female copulates rarely during their life cycle, at the same time involved in multiple breeding.

An electrophoretic analysis of the male reproductive tissues of *P. plicatum* has made to study the origin of different proteins from the reproductive tissues. In the present study, a single peptide fraction was resolved in the testis and ten were resolved from vas deferens and accessory gland. The electrophoretic data obtained in the present study revealed that 58 kDa protein fraction is common to vas deferens and accessory gland. We presume that the presence of identical peptides may be because the whole posterior vas deferens fringed with accessory glands and there is every chance of mixing up of these two secretions at their junctions. Moreover, separation of accessory gland tubules from posterior vas deferens is quite difficult. On the other hand, the epithelium of vas deferens and accessory gland secretes similar peptides, which may have an important role in copulation and thereby succeeding fertilization. Although the potential roles of the ductal gland secretions are considerable and stressed that a complete chemical characterization of the vas deferens and accessory gland secretion would be necessary for a full understanding of their roles in reproduction.

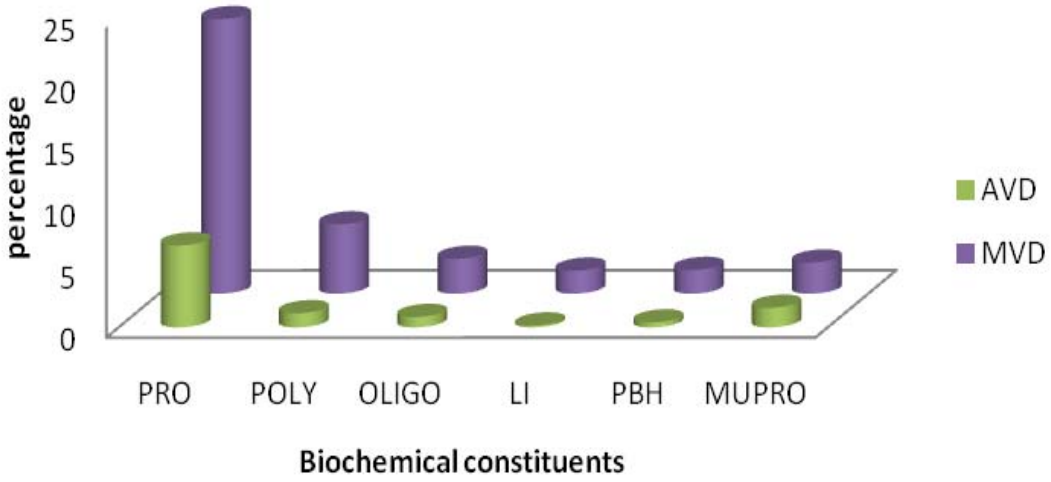
Table. 1. Biochemical composition of testis, vas deferens and accessory gland of *P.plicatum*

Organic Constituents	Weight shown in mg/100 mg		
	Testis	VD	AG
Total Protein	3.94 ± 1.16	28.62 ± 4.12	21.6 ±2.71
Polysaccharide fractions	1.39 ±0.47	4.6 ±0.64	2.48 ±0.77
Oligosaccharide fractions	0.8 ±0.24	2.82 ±0.28	1.68 ±0.26
Protein-bound hexose	0.32 ±0.17	1.92 ±0.43	1.06 ±0.28
Mucoprotein	0.48 ±0.25	2.52 ±0.29	1.62 ±0.31
Glycogen	Traces	0.92 ±0.28	0.28 ±0.16
Glucose	Traces	0.07 ±0.11	0.04 ±0.03
Ascorbic acid	Traces	0.04 ±0.01	0.024 ±0.008
Lipid	0.84 ±0.17	1.76 ±0.13	0.9 ±0.07

Table.2 Biochemical composition of testis, vasdeferens and accessory gland of *P.plicatum*

Inorganic Constituents	Weight shown in m Eq/100 mg		
	Testis	VD	AG
Sodium	0.85 ±0.43	6.3 ±1.4	2.76 ±0.68
Potassium	0.21 ±0.01	0.53 ±0.04	0.29 ±0.03
Magnesium	0.18 ±0.03	1.2 ±0.04	0.49 ±0.04
Calcium(%)	0.11 ±0.09	0.34 ±0.03	0.22 ±0.02

Fig. 1. Biochemical constituents of AVD and MVD of *P.plicatum*



PRO - Protein, POLY - Polysaccharide, OLIGO - Oligosaccharide
LI - Lipid, MUPRO- Mucoprotein

Fig.3. Densitogram of SDS-PAGE of standard protein fractions

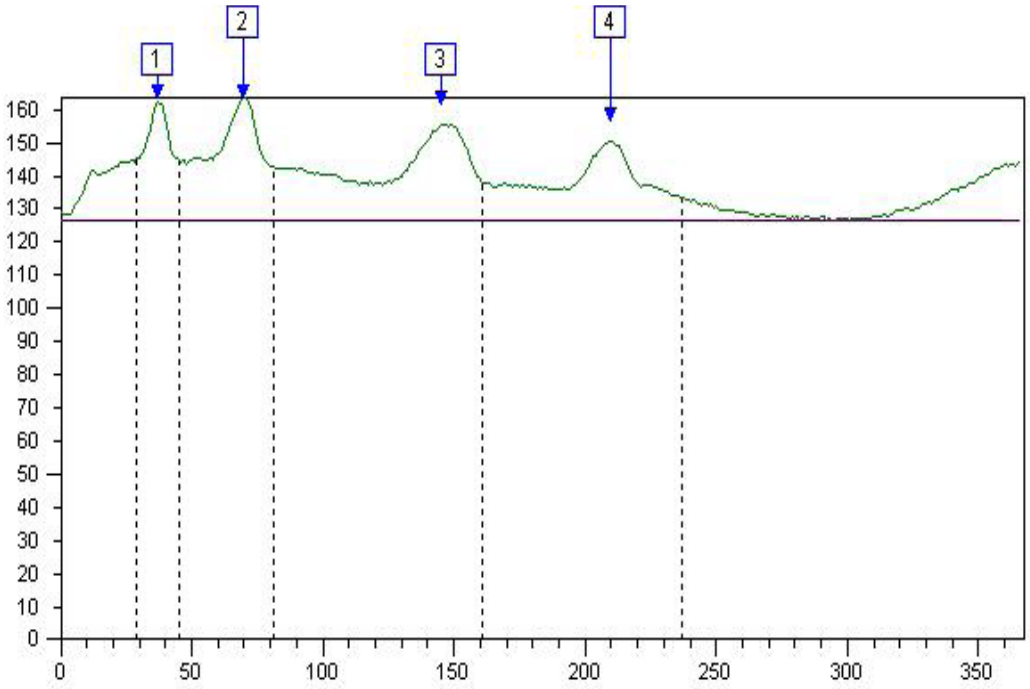


Table 3. Description of Densitogram

Band No	Volume	Vol+BkGnd	Calib Vol(ug)	Norm'd Vol	MW (kd)	Rf
1	29,077.00	170,949.00	205,000.00	0.00	205,000.000	0.101
2	58,758.00	377,970.00	-	0.00	97,400.000	0.191
3	92,619.00	801,979.00	-	0.00	66,000.000	0.396
4	65,805.00	739,697.00	-	0.00	43,000.000	0.574

Fig..6. Densitogram of SDS-PAGE of Accessory glands of *P. plicatum*

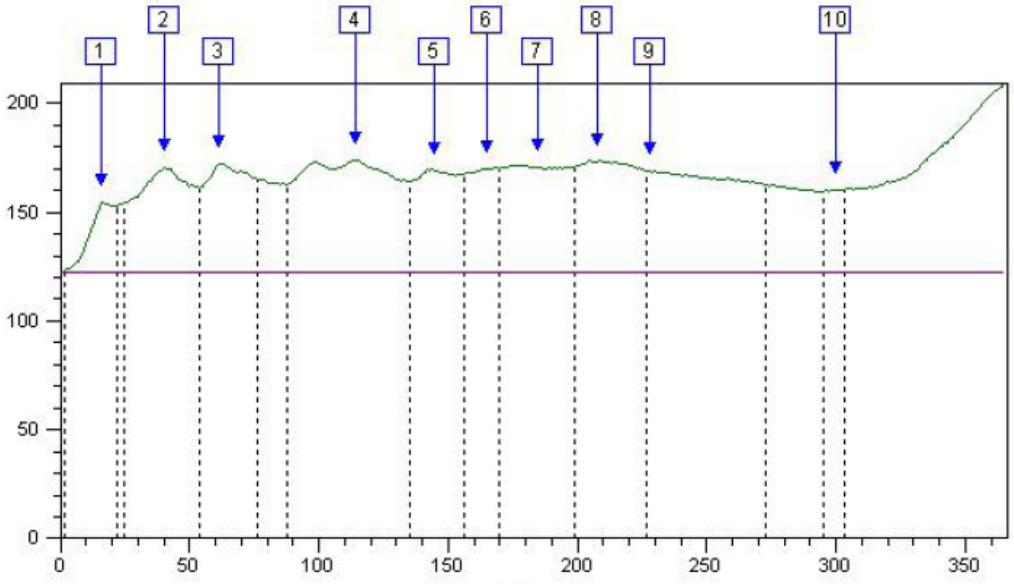


Table 4. Description of Densitogram

Band No	Volume	Vol+BkGnd	Calib Vol(ug)	Norm'd Vol	MW (kd)	Rf
1	28,764.00	228,964.00	-	0.00	273,472.727	0.044
2	97,910.00	388,200.00	-	0.00	193,800.898	0.110
3	82,723.00	302,943.00	-	0.00	121,359.776	0.167
4	181,897.00	652,367.00	-	0.00	58,915.024	0.312
5	77,954.00	288,164.00	-	0.00	58,915.024	0.397
6	54,002.00	194,142.00	-	0.00	58,915.024	0.452
7	115,415.00	405,705.00	-	0.00	56,484.099	0.507
8	114,527.00	394,807.00	-	0.00	43,594.910	0.570
9	165,558.00	626,018.00	-	0.00	36,171.875	0.625
10	24,848.00	104,928.00	-	0.00	10,296.875	0.822

Fig..4. Densitogram of SDS-PAGE of Testis of *P. plicatum*

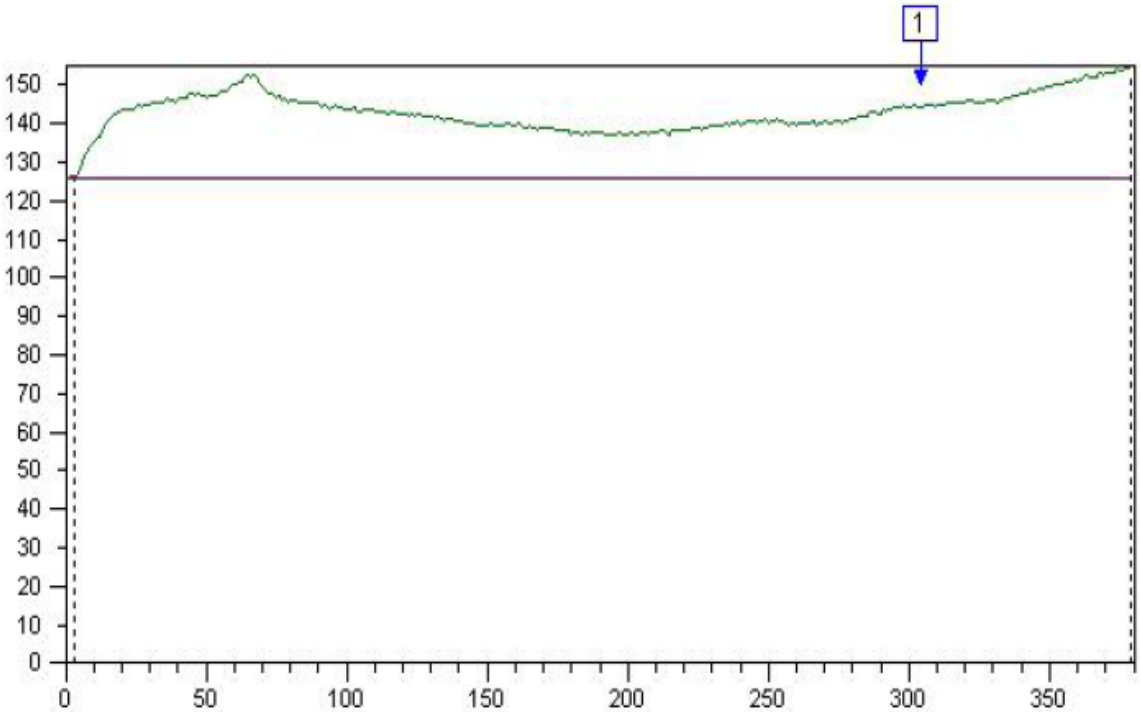


Table 5. Description of Densitogram

Band No	Volume	Vol+BkGnd	Calib Vol(ug)	Norm'd Vol	MW (kd)	Rf
1	473,924.00	3,982,380.00	-	0.00	13,134.328	0.802

Fig..5. Densitogram of SDS-PAGE of Vas deferens of *P.plicatum*

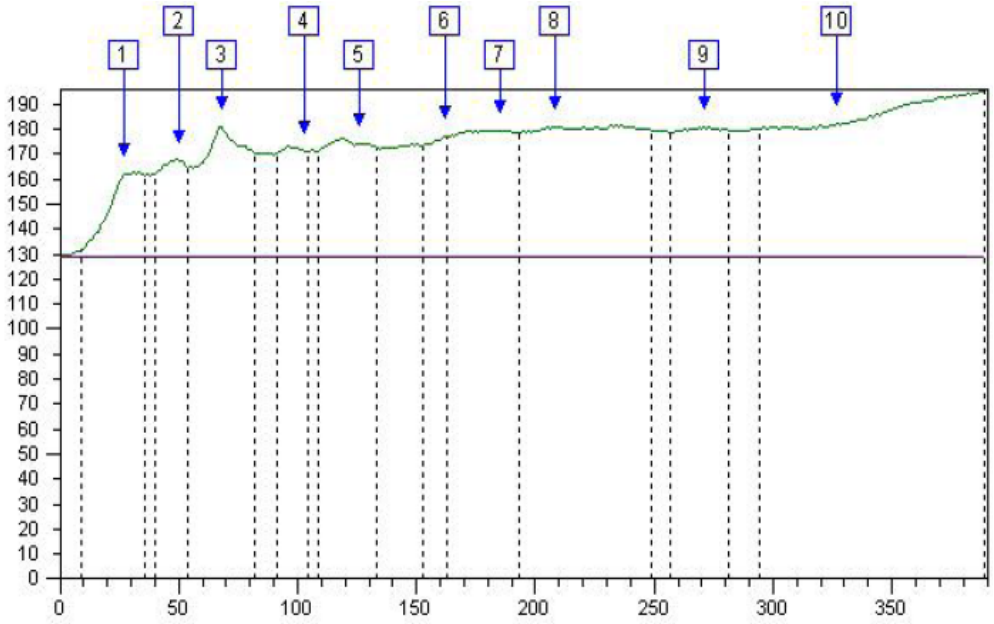


Table 6. Description of Densitogram

Band No	Volume	Vol+BkGnd	Calib Vol(ug)	Norm'd Vol	MW (kd)	Rf
1	42,346.00	307,216.00	-	0.00	241,891.429	0.069
2	38,922.00	176,262.00	-	0.00	166,746.945	0.129
3	91,656.00	366,336.00	-	0.00	111,778.001	0.175
4	42,288.00	169,818.00	-	0.00	61,570.914	0.265
5	82,179.00	317,619.00	-	0.00	58,684.529	0.324
6	34,918.00	133,018.00	-	0.00	58,684.529	0.416
7	113,262.00	407,562.00	-	0.00	58,684.529	0.476
8	217,183.00	766,543.00	-	0.00	51,188.589	0.535
9	92,611.00	328,051.00	-	0.00	27,000.000	0.697
10	411,294.00	1,343,244.00	-	0.00	8,333.333	0.841

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