International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Effect of Fabric Dyes and Metal Surfaces on DNA Amplification

Salahudeen Alrabie[a], Maryam Yaqoob[b], Hamda Turkiah[c], Jawad Salehi[d], Sheikha Sanqoor[e], Venkata Lakshmi[f], Muhammad Nazir[g]

[a,b,d,f,g]*College of Biotechnology, University of Modern Sciences, United Arab Emirates*

[c,e]*Gen. Dep. of Forensic Science & Criminology, Dubai Police, UAE*

[a]*Email: salah_246@outlook.com,* [b]*Email: maryam.yy.92@gmail.com,* [c]*Email: H_bin.turkiah@hotmail.com*

[d]*Email: j.salehi@ums.ae,* [e]*Email: sheikhassss@yahoo.com ,* [f]*Email: lakshmi.tetali@yahoo.com*

[g]*Email: m.nazir@ums.ae*

Abstract

Cloths and metal objects are material evidences frequently found on crime scenes and can provide valuable information hidden in the form of DNA, either perpetrator's or victims'. DNA profiling is an important aspect of forensic investigation and requires extraction and amplification of the DNA from evidence. These procedures can be affected by the chemical and physical properties of the evidence. For example, synthetic dye molecules such as SYBR Green I is known to intercalate with DNA and show PCR inhibition when present above certain threshold concentration. On the other hand, chemical interactions between metal ions and specific binding sites on DNA can strongly affect DNA's structure and stability. The metal ions can also result in PCR inhibition. In this study, the effect of five different coloured cloths (white, black, red, blue and green) and five different metal surfaces (iron, rusty iron, stainless steel, lead and copper) on DNA extraction and amplification was assessed. DNA quantification was performed using CFX96 Touch™ Real Time PCR Detection System (BioRad®) after PCR amplification using RAG-1 primers. Results revealed that different metal surfaces and fabric dyes had no effect on PCR amplification, eliminating the probability of inhibitors present due to use of DNA extraction kits. Future research should be focused on the relationship of different variables influencing DNA persistence on different types of hard surfaces such as wood and glass and also clothes with different colors and composition.

Keywords: Forensic Science; DNA Evidence; PCR inhibition.

---

* Corresponding author.

1. Introduction

DNA evidence plays a vital role in solving criminal cases globally, both to convict the guilty and exonerate the innocent. Biological samples that were impossible to test for DNA analysis earlier, may yield critical evidence if tested today due to advances in DNA extraction and amplification technologies [1]. DNA profiling is a well-established scientific technique, used worldwide for criminal intelligence, paternity testing and mass disaster victim identification. This technique makes use of repetitive sequences that are highly variable between individuals. It was first described in 1985 using variable number tandem repeats (VNTRs) but is now most commonly achieved using short tandem repeats (STRs) [2].The main aim of forensic committee is to obtain sufficient quantity of DNA to generate a complete DNA profile. However, studies have shown that not only the pre-collection conditions but also the post-collection storage conditions may affect the quality and quantity of DNA recovered from a specimen [3]. Moreover, several other factors could affect the quality of recovered DNA sample such as exposure to sun light, microbial contamination, high temperature and humidity. DNA samples that are subjected to such environmental factors which promote degradation can pose challenges for data interpretation, since STR regions of the DNA molecule can be fractured leading to ineffective amplification results. Larger STR loci are more prone to degradation compared to their smaller counterparts. Polymerase chain reaction (PCR) enables researchers to produce millions of copies of a specific DNA sequence while PCR inhibitors tend to generally exert their effect through direct interaction with DNA or thermostable DNA polymerases. Direct binding of agents to single stranded or double-stranded DNA can prevent amplification and facilitate co-purification of inhibitor and DNA [4].

Dyes are coloured substances that have an affinity to the substrate to which they are being applied. One type of crime scene evidence commonly submitted for analysis is bloodstain on denim. However dyes such as indigo may co-purify with DNA and affect DNA analysis [5]. Dye induced PCR inhibition as described by a delay in Ct, is probably because of the interference of dye chemicals with the chain extension step, where dye can bind to the single-stranded template, thus preventing the extension of primer-template complex. Therefore, an ideal concentration of dye should have as little interaction as possible with ssDNA [6]. The affinity of dye depends on the substances being content within such as textile dyes from clothing or haemoglobin from red blood cells may also contain DNA polymerase inhibitors and can remain with the DNA throughout the sample preparation process and interfere with the polymerase to prevent successful PCR amplification [7]. The results of amplifying a DNA sample containing an inhibitor, such as hematin, results in loss of the alleles from the larger-sized STR loci or even complete failure of all loci. Extensive research have been performed about the DNA recovery from different substrates and their effects on extraction [8, 9]. Recent research conducted in 2013 examined the effect of substrate on DNA transfer and extraction efficiency by testing variable of nine substrates commonly found in crime scenes (acetate, polyester, calico, poly/cotton, flannelette, tarpaulin, plywood, plastic and cotton drill) and results have shown that quality and quantity of DNA extracted from these substrates vary. All white colored fabrics were tests in order to avoid dye induced PCR inhibition [10].

This study examined the effect of five different fabric dyes and metal surfaces on DNA amplification. It was expected that different dyes can have a major effect on DNA recovery and PCR amplification due to their differing properties. Concurrently, different metals surfaces (copper, lead, stainless steel, iron and rusty iron)

were used to determine the effect of metal surfaces on DNA amplification since such metals are being commonly used in different crimes. The main aim of this study was to investigate the effect of both, metals surfaces and textile dyes, in DNA recovery and amplification.

2. Material and Methods

2.1. Experimental Design

Experimental samples consisted of five different colors: white, black, red, blue and green having same composition (65% polyester and 35% cotton); from the same brand (Gul Ahmed, Pakistan). A total of 48 bloodstain samples (2 cm x 2 cm) were prepared by depositing 200 µL of fresh blood from a male volunteer on each of the five colors of cotton material in triplicate sets, and left at room temperature (20^{o}C-22^{o}C) away from direct sunlight for 21 days. Cloths were chosen suiting the hot climate and thus more commonly worn by UAE population.

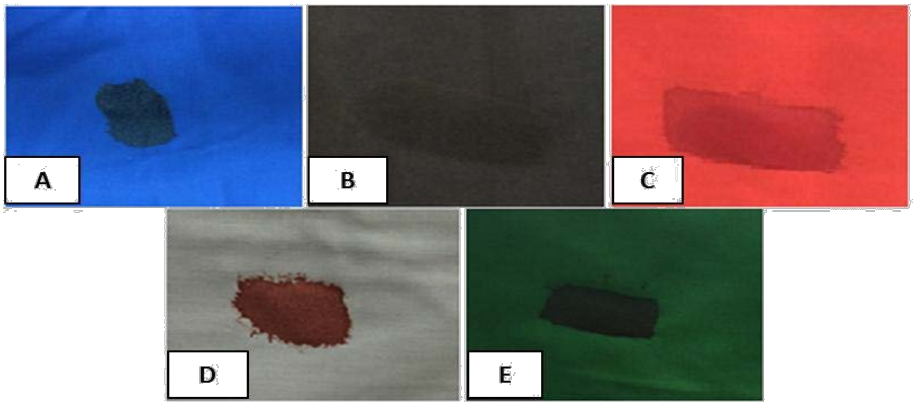

Figure 1: Shows different coloured fabrics with bloodstains: A) Blue, B) Black, C) Red, D)White and E) Green.

Another set of experimental samples consisted of five metals: Iron, lead, copper, rusty iron, and stainless steel. A total of 48 samples were prepared by depositing 200 µL blood in triplicate sets for each surface. The samples were incubated for 21 days at room temperature away from sunlight.

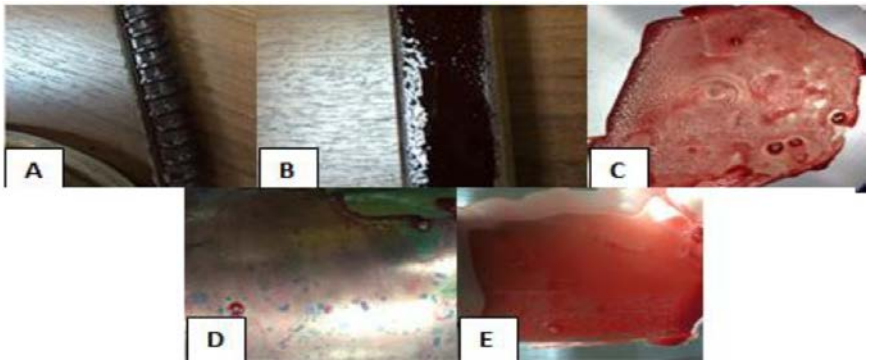

Figure 2: Shows different metal surfaces with bloodstains. A) Iron, B) Aluminium, C) Copper, D) Stainless steel, E) Rusty iron.

2.2. Sample Collection

Triplicate samples of blood deposited area were collected from each colored fabric at four different time points by cutting (1 cm^2) area of the fabric. Blood on metal surfaces were swabbed with sterile cotton swabs. The samples both from fabrics and metal surfaces were collected at day zero, day 7, day 14 and day 21 to determine the possible effects of time in correlation with dye color/metal surface.

2.3. DNA Extraction

DNA was extracted using Genomic DNA Isolation Kit (NORGEN Biotek CORP, Canada) according to the manufacturer's protocol. Cloth and cotton swabs samples containing blood were put separately in Eppendorf tubes. 300 µl cell lysis solution and 12 µl proteinase K was added to the samples. The samples were then vortexed and incubated at 56°C for 2 hours. After the incubation, 300 µL of binding solution was added to the lysate and vortexed. Next, the lysate was added to the micro spin column assembled with the collection tube, which was then centrifuged for 3 minutes at 5,200 x g (~ 8,000 RPM). The spin columns were then washed twice using 500 µL wash solution by centrifuging the tubes for 1 minute at 14,000 x g (~14,000 RPM). DNA was then eluted using 200 µl of elution buffer and centrifuging for 1 minute at 3,000 x g (~6,000 RPM). The extracted DNA was stored at -20 ^oC until further analysis.

2.4. PCR Optimization

The PCR conditions were optimized at different temperatures using different concentrations of DNA template, primers and concentration of $MgCl_2$ to reach the most optimum conditions. PCR reaction was set up by adding 7.5 µl of master mix, 0.25 µl of both forward and reverse primers, 1 uL of extracted DNA sample, and 6 µl of molecular grade water. PCR conditions used are presented in Table 2: Initial denaturation at 94^oC for 3 minutes, denaturation at 94^oC for 1 minute, annealing at 60^oC for 1 minute, extension at 72^oC 1 minute, and final extension at 72^oC for 10 minutes; for 33 cycles.

Table 1: RAG-1primer details [11].

Primer	Annealing temperature (^oC)	Forward (5'-3') sequence	Reverse (5'-3') sequence
RAG-1	60	TCCAACCATCACCCAACAGTC	TGAGTCCCATTACGGGTCCAG

2.5. DNA Quality

The quality of the extracted DNA samples was assessed using agarose gel electrophoresis. An agarose gel of

1.5% (w/v) in an electrophoresis gel tank (12 cm x 6 cm) was used. DNA samples were prepared by mixing 7 µl of sample with 3 µl of loading dye. The gel was run at 80 V for 60 minutes, stained in 0.5 µg/ml ethidium bromide and visualized using a UV trans-illuminator (Bio Doc-It™ imaging system, USA).

Table 2: Polymerase chain reaction conditions.

Stage	Temperature (°C)	Time	Cycles
Initial Denaturation	94	3 minutes	1
Denaturation	93	1 minute	
Annealing	60	1 minute	33
Extension	72	1 minute	
Final Extension	72	10 minutes	1

2.6. DNA Quantification

Samples were quantified using CFX96 Touch™ Real Time PCR Detection System (BioRad®). The differences in DNA quantity were assessed based on the melting curve and level of fluorescence of both SYBR® Green I and SYBR® Green ER™ dyes. Serial dilutions were prepared in 8 series from 100 ng human standard DNA stock starting from 20 ng to 0.15 ng. Complete 96 wells plate Real Time PCR reaction was carried out using standard human DNA, positive control, negative control and DNA samples extracted from 5 colored fabrics taken on 4 time points in addition to DNA samples extracted from 5 metal surfaces taken on 4 time points.

3. Results and Discussion

3.1. DNA Quantification

DNA amplification of RAG-1 primers was obtained in all samples of fabric and metal surfaces.

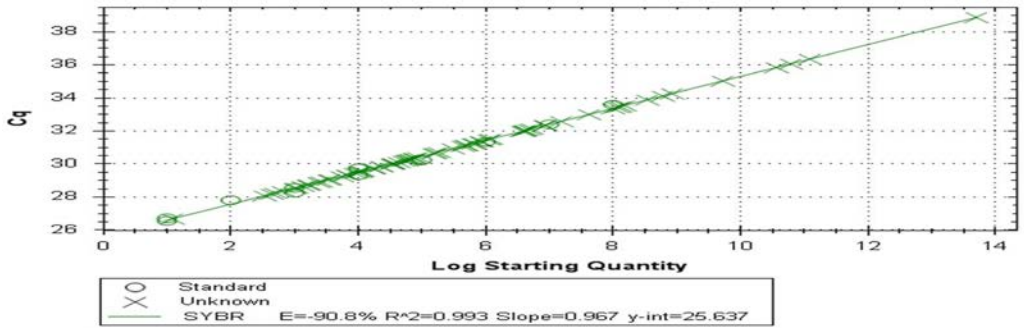

Figure 3: Shows a standard curve using known quantity human DNA standard.

DNA quantity varied significantly among various colors with notable change at different time points. After the

deposition of blood on day zero, there was gradual rise and fall in DNA quantity extracted during the 21 days period. DNA quantity after two hours exposure on day zero was the least among four time points in the red, white and green fabrics. It started increasing on the next time point (Day 7) for the same colors and further increased gradually over time in green fabric (Day 14 and 21). However, it slightly declined after two weeks for red colored fabric and remained constant for white fabric. DNA quantity varied frequently especially in blue and black fabric through four time points in all triplicates. Interestingly, higher DNA quantity was o from blood deposits on blue, black, red and white fabrics on day 14. Our results show that the quantity of DNA extracted from blood stained fabrics and metals vary at different time points. The main reason for this could be the chemical and physical properties of fabrics and metals used in this study. Dyes chemicals can interfere with the chain extension step, where dye can bind to the single-stranded template, thus preventing the extension of primer-template complex. Therefore, an ideal concentration of dye should have as little interaction as possible with ssDNA [6]. For example, fabrics and metals contain functional groups that could possibly lead to the formation of hydrogen bonds and dipole–dipole attractions between DNA molecules and fabrics as well as metals. Moreover, the hydroxyl groups present in cotton are capable to make strong hydrogen bonding with DNA molecules that result in strong intermolecular attractions while the carbonyl (CMO) and cyano (CQN) groups of polyester allow relatively weaker dipole–dipole attraction with the DNA molecules and as result we see variation in the quantity of DNA extracted at different time points. Seah and his colleagues showed the extraction of high quality DNA from cotton fabrics at day 30.This is comparable to our results where we have been able to extract high quality DNA from blood stained fabrics (65% polyester and 35% cotton) at day 21. However, our results indicate a decline in the quantity of DNA extracted from red colored fabrics after two weeks whereas no variation is seen in the quantity of DNA extracted from white colored fabrics. The possible explanation for this could be the chemical nature of red and white colors that may or may not interact with DNA molecules [12].

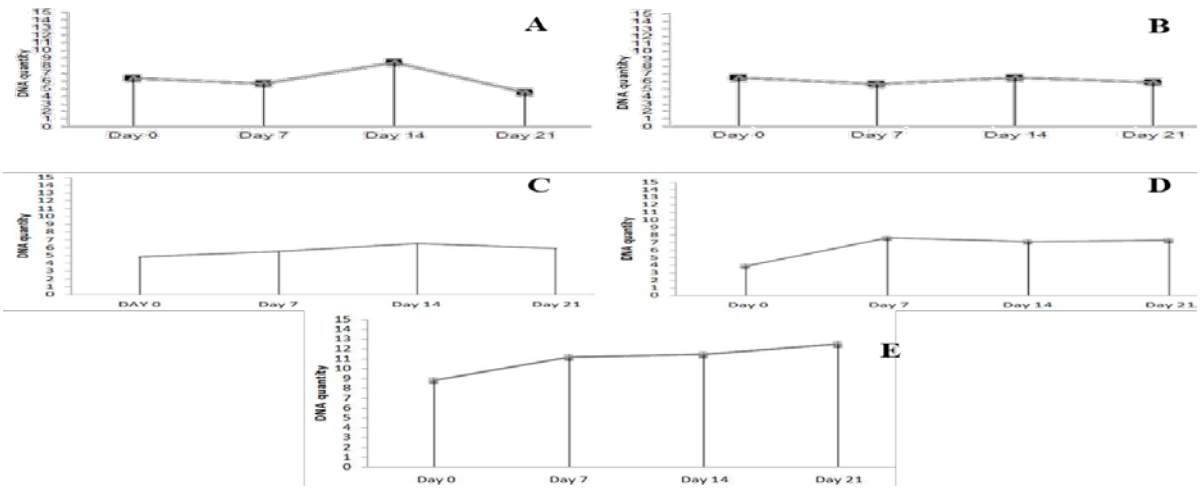

Figure 4: Shows DNA quantity for different colored fabric samples through 21 days period. A) Blue, B) Black, C) Red, D) White, E) Green.

3.2. Metal Surfaces

Iron, stainless steel and rusty iron showed highest DNA quantity on day zero with significant decline through three weeks since DNA degraded along with metal ions being intercalated over time. Surprisingly, significant rise in the DNA quantity for iron sample was observed after second week. Copper sample showed gradual increase in DNA quantity till day 14; however it then declined the following week. Aluminum sample fluctuated throughout the 21 days period giving highest DNA quantity at the last week. Our results are in concordance with previous studies which show that the reaction of metal ions can have deleterious effects on quality of nucleic acids leading to a variety of dramatic effects on nucleic acid structure, e.g., crosslinking of the polymer strands, degradation to oligomers and monomers, stabilization or destabilization, and the mispairing of bases [13].

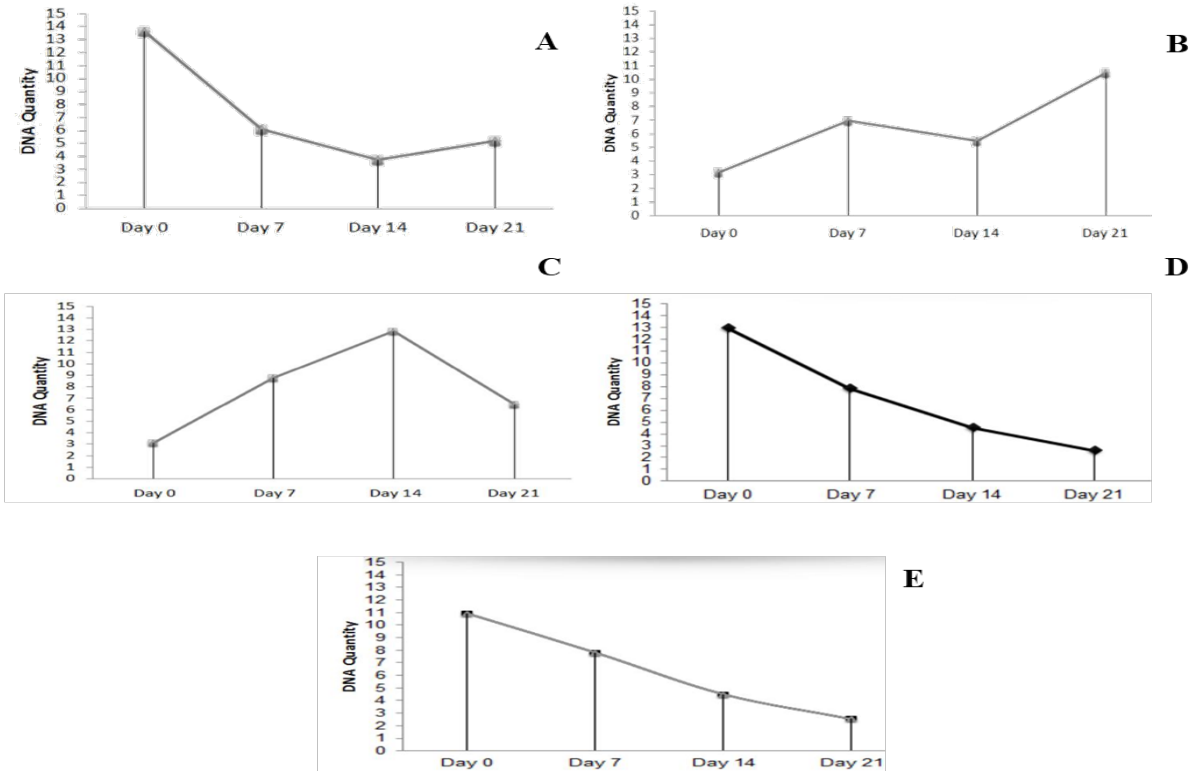

Figure 5: Shows DNA quantity for different metal surface samples through 21 days period. A) Iron, B) Aluminum, C) Copper, D) Stainless steel, E) Rusty iron.

4. Conclusion

This study examined the effect of five fabric dyes and five metal surfaces on DNA amplification. Resultsrevealed that different metal surfaces and fabric dyes had no effect on PCR amplification, eliminating the probability of inhibitors present due to use of Norgen Biotech corp. Kit in DNA extraction which eliminated the inhibitors. However, findings show that substrate type or color can have a major effect on DNA recovery due to their differing properties, the chemical composition, the weaves and the thickness of fabrics keeping into consideration fabrics type was identical in this study. More research is needed to study the interaction of forensically relevant biological materials with various substrates and different colors, leading to identification and a greater understanding of the mechanisms which underpin the DNA persistence in sufficient quality and quantity.

5. Future work

Future research should focus on the relationship of other variables influencing DNA persistence on different types of hard surfaces such as wood and glass, also clothes and other fabric colors.

6. Conflict of interest

None.

References

[1] K.M. Turman. “Understanding DNA Evidence: A Guide for Victim Service Providers.” Office of Justice Programs. 2001. Retrieved on June, 30, 2013.

[2] A.J. Jeffreys, V. Wilson, S.L. Thein. “Hypervariable 'minisatellite' regions in human DNA”. Nature, 314, pp.67-73, 1985.

[3] L. Koblinsky. “Recovery and stability of DNA in samples of forensic science significance.” Forensic Sci. Rev, 4(1), pp.68–87, 1992.

[4] J. Bessetti. “An introduction to PCR inhibitors.” Profiles in DNA, 10(1), pp.9–10, 2007.

[5] B.H. Perdigon, C.G. Calacal, L.K. Co Seng, C.S. Halos, A.M. De Ungria. “Evaluation and In-House Validation of Five DNA Extraction Methods for PCR-based STR Analysis of Bloodstained Denims.” Science Diliman, 16(1), pp.37-38, 2004.

[6] F. Mao, W.Y. Leung, X. Xin. “Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications.” BMC Biotechnology, 7, pp.76, 2007.

[7] P. Rådström, R. Knutsson, P. Wolffs, M. Lövenklev, C. Löfström. “Pre-PCR processing: Strategies to generate PCR-compatible samples.” Molecular Biotechnology, 26, pp.133–146, 2004.

[8] P. Neubauer, B. Wrobel, G. Wegrzyn. “DNA degradation at elevated temperatures after plasmid amplification in amino acid-starved Escherichia coli cells.” Biotechnology letters, 18(3), pp.321-326, 1996.

[9] J.I. Sheu, E.Y.Sheu. “Characterization of DNA degradation using direct current conductivity and dynamic dielectric relaxation techniques.” AAPS PharmSciTech, 7(2), pp.E38-E44, 2006.

[10] T.J. Verdon, R. Mitchell, R.A. Oorschot. “The influence of substrate on DNA transfer and extraction efficiency.” Forensic Science International: Genetics, 7(1): pp.167–175, 2013.

[11] M.S. Nazir, S. Iyavoo, S. Alimat, N. Zahra, S.H. Sanqoor, J.A. Smith, C. Moffatt, W. Goodwin. “Development of a multiplex system to assess DNA persistence in taphonomic studies.” Electrophoresis, 34(24), pp.3352-60, 2013.

[12] L.H. Seah, M.I. Othman, P. Jaya, N.H. Jeevan. “DNA profiling on fabrics: an in-situ method.” International Congress Series, 1261, pp.565– 567, 2004.

[13] J.J. Butzow, G.L. Eichhorn. “Different susceptibility of DNA and RNA to cleavage by metal ions”. Nature, 254, pp.358 – 359, 1975